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Role of *vif* in Replication of Human Immunodeficiency Virus Type 1 in CD4+ T Lymphocytes

DANA H. GABUZDA,[1,2] KATHARINE LAWRENCE,[1] ERIK LANGHOFF,[1,3] ERNEST TERWILLIGER,[1,3] TATYANA DORFMAN,[1,3] WILLIAM A. HASELTINE,[1,3] AND JOSEPH SODROSKI[1,3]*

Division of Human Retrovirology, Dana-Farber Cancer Institute, 44 Binney Street,[1] and Departments of Neurology[2] and Pathology,[3] Harvard Medical School, Boston, Massachusetts 02115

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The viral infectivity factor gene *vif* of human immunodeficiency virus type 1 has been shown to affect the infectivity but not the production of virus particles. In this study, the effect of *vif* in the context of the HXB2 virus on virus replication in several CD4+ T-cell lines was investigated. *vif* was found to be required for replication in the CD4+ T-cell lines CEM and H9 as well as in peripheral blood T lymphocytes. *vif* was not required for replication in the SupT1, C8166, and Jurkat T-cell lines. The infectivity of *vif*-defective viruses depended on the cell type in which the virus was produced. In CEM cells, *vif* was required for production of virus capable of initiating infection in all cell lines studied. *vif*-defective virus produced by SupT1, C8166, and Jurkat cells and the monkey cell line COS-1 could initiate infection in multiple cell lines, including CEM and H9. These results suggest that *vif* can compensate for cellular factors required for production of infectious virus particles that are present in some cell lines such as SupT1, C8166, and Jurkat but are absent in others such as CEM and H9 as well as peripheral blood T lymphocytes. The effect of *vif* was not altered by deletion of the carboxyl terminus of gp41, a proposed target for *vif* (B. Guy, M. Geist, K. Dott, D. Spehner, M.-P. Kieny, and J.-P. Lecocq, J. Virol. 65:1325–1331, 1991). These studies demonstrate that *vif* enhances viral infectivity during virus production and also suggest that *vif* is likely to be important for natural infections.

The *vif* protein of human immunodeficiency virus type 1 (HIV-1) is an important determinant of viral infectivity (7, 29). In some experiments *vif* is reported to increase the infectivity of HIV-1 particles as much as 100- to 1,000-fold (7, 29) and may also enhance cell-to-cell virus transmission (7, 22). The presence of *vif* antibodies in sera of patients at all stages of HIV-1 infection indicates that *vif* is expressed during natural infections in vivo (2, 13, 15). A *vif* open reading frame is found in other lentiviruses, including HIV-2, simian immunodeficiency virus, visna virus, caprine arthritis-encephalitis virus, and feline immunodeficiency virus (3, 17, 28, 30). These observations suggest that *vif* plays an essential role during natural infections.

The 23-kDa *vif* protein is encoded by a singly spliced 5-kb transcript. *rev* function is required for *vif* expression (10, 24). *vif* is present in infected cells but is not associated with the mature virus particle (2, 7, 13, 15, 22, 27). The results of previous studies indicated that the requirement for *vif* differs among different cell lines (7, 16, 27, 29). *vif* does not have a major effect on transcription, translation, or virus release (7, 27, 29). The *rev* dependence and expression of *vif* mRNA late in the virus life cycle together with the *gag*, *pol*, and *env* mRNAs suggest that *vif* may be important during late events in the virus life cycle involved in the processing or assembly of viral structural proteins. It has been proposed that *vif* may play a role in the processing or conformation of the HIV-1 envelope glycoproteins (11).

The goal of the present study was to determine whether *vif* must be present when the virus particle is assembled or whether *vif* is required during the establishment of infection. The studies also investigate the role of *vif* during virus replication in several different cell lines and in peripheral blood T lymphocytes.

* Corresponding author.

MATERIALS AND METHODS

Plasmids. The pHXB2 plasmid contains the infectious viral DNA clone HXB2 that originates from the HIV-1 IIIB isolate (17, 20). The *vif* mutant of HXB2, HXBΔAvr, was created by removing nucleotides 5020 to 5240 between two *Avr*II restriction sites to create the pHXB2ΔAvr plasmid. This deletion removes the 3′ coding sequences of *vif* and the 5′ coding sequences of *vpr* which overlap *vif* (27). This deletion results in the same phenotype as a deletion which encompasses nearly the entire *vif* gene (27). The HXBΔAvr *vif* mutant was used for all experiments unless otherwise noted. An additional *vif* mutant of HXB2, HXB2*vif*stop, was made by changing the sequence encoding *vif* amino acids 21 and 22 (TGG AAA) to two in-frame stop codons (TAG TAA) by using the polymerase chain reaction method and confirmed by DNA sequencing. These nucleotide substitutions result in a truncated 20-amino-acid *vif* protein. The pHXBΔenvCAT plasmid contains an HIV-1 provirus with an in-frame deletion from the *Bgl*II to *Bgl*II sites (nucleotides 6620 and 7200) of the sequence identified by Ratner et al. (20) in the *env* gene and a chloramphenicol acetyltransferase (CAT) gene replacing the *nef* gene (12, 31). The pHXBΔAvrΔenvCAT plasmid was made by creating the *Avr*II-to-*Avr*II deletion in the pHXBΔenvCAT plasmid. The pSVIIIenv plasmid expresses the HIV-1 *env* and *rev* genes of the HXB2 strain under the control of the HIV-1 long terminal repeat (12).

Transfection of T-cell lines. The DEAE-dextran method (19) was used to transfect 10 μg of *vif*-positive or *vif*-negative HXB2 DNA into 10^7 cells. The transfected cultures were maintained in RPMI 1640 plus 10% fetal calf serum, with daily medium changes. Reverse transcriptase activity of pelleted virions was measured as previously described (21).

Virus stocks. *vif*-positive and *vif*-negative HXB2 virus stocks produced in the SupT1, C8166, and Jurkat cells were prepared from supernatants of the cultures transfected with 10 μg of the *vif*-positive or *vif*-negative HXB2 DNAs. When peak levels of reverse transcriptase were achieved (usually day 11 or 13), virus stocks were prepared by centrifugation and filtration (0.45-μm pore size) to remove cell debris and dilution at 1:1 with fetal calf serum and stored at -70°C. To prepare *vif*-positive and *vif*-negative virus stocks produced in CEM cells, infection of 5×10^6 CEM cells was initiated with 20,000 cpm of reverse transcriptase units (approximately 0.2 infectious unit per cell) of *vif*-positive and *vif*-negative virus originally produced in SupT1 cells.

Infection of peripheral blood T lymphocytes. Peripheral blood mononuclear cells were isolated by separation on Ficoll from buffy coats obtained from healthy seronegative donors (14). The T-cell fraction (97% CD2 positive) was high-density cells from a Percoll gradient rosetted with neuroaminidase-treated sheep erythrocytes as described previously (14). The cultures were grown in RPMI 1640 with 10% fetal calf serum, antibiotics, and 20% phytohemagglutinin-conditioned medium made in our laboratory (14). Cultures of peripheral blood T cells were infected with equivalent amounts of virus stock (5,000 cpm of reverse transcriptase units) for 16 h. Following infection, the medium was completely replaced each day for 2 days and thereafter every third day. Virus replication was monitored by measuring viral p24 antigen production in the culture supernatants every third day immediately before medium replacement by using a radioimmunoassay kit (Dupont/NEN).

Replication complementation assay. The *trans*-complementation assay used to measure the efficiency of both cell-free and cell-to-cell transmission during a single round of virus replication has been previously described (12). The T-cell lines (10^7 cells) were transfected with 5 μg of pHXBΔenvCAT or pHXBΔAvrΔenvCAT and 5 μg of pSV-IIIenv. The efficiency of a single round of infection was measured by assaying for CAT activity in the transfected culture 9 days after transfection. A similar *trans*-complementation assay was used to measure the cell-free transmission of virus produced in COS-1 cells (12). COS-1 cells were transfected with 5 μg of the HIV-1 envelope expressor plasmid and 5 μg of either the *vif*-positive or *vif*-negative envelope-defective CAT virus DNA by the DEAE-dextran method (4, 12). At 48 to 72 h after transfection, the COS-1 cell supernatants were filtered (0.45-μm pore size) and the amount of virus in the supernatant was determined by measurement of reverse transcriptase activity (21). Equivalent amounts of *vif*-positive or *vif*-negative CAT virus (5,000 or 10,000 cpm) were added to 5×10^6 CD4$^+$ target cells. The infected target cells were incubated for 48 to 72 h, and the level of CAT enzyme activity was measured (12).

Radioimmunoprecipitation. Jurkat and SupT1 cultures (5×10^6 cells) were metabolically labelled with 100 μCi each of [^{35}S]cysteine and [^{35}S]methionine per ml for 16 h. The labelled cells were lysed in radioimmunoprecipitation assay lysis buffer (0.15 M NaCl, 0.05 M Tris-HCl [pH 7.2], 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate) at 0°C and immunoprecipitated as previously described (12).

TABLE 1. Effect of *vif* on a single round of HIV replication in different T-cell lines

Cell line	Relative CD4 expression on the cell surface[a]	Relative syncytium-forming ability[b]	CAT activity (fold enhancement) during: Virus replication[c]	CAT activity (fold enhancement) during: Cell-free transmission of viruses from COS cells[d]
SupT1	1.0	1.0	1.8	1.0
C8166	0.35	0.48	2.0	ND[e]
Jurkat	0.21	0.82	3.1	1.0
CEM	0.29	0.03	3.4	1.0
H9	0.13	0.12	6.1	1.0

[a] Values shown represent the mean fluorescence intensity of CD4 expressed on the cell surface relative to that of SupT1 cells. The mean fluorescence intensity was determined by using a monoclonal antibody against CD4 (MAX.16H5) (6) and calculating ratios of specific fluorescence intensities by FACS analysis as previously described (32).

[b] Values shown represent the syncytium-forming abilities of the different T-cell lines relative to that of SupT1 cells. COS-1 cells were transfected with 5 μg of the HIV-1 envelope expressor plasmid and cocultured with a 10-fold excess of SupT1, C8166, Jurkat, CEM, or H9 cells for 16 h at 60 h posttransfection. The number of syncytia formed were scored by counting.

[c] Values shown represent the fold enhancement of CAT activity during a single round of transmission of the *vif*-positive CAT virus relative to that of the *vif*-negative CAT virus following cotransfection of the HIV-1 envelope expressor plasmid and the *vif*-positive or *vif*-negative envelope-defective CAT virus DNA directly into each T-cell line (12).

[d] Values shown represent the fold enhancement of CAT activity following a single round of cell-free transmission of *vif*-positive relative to *vif*-negative recombinant virions produced in COS-1 cells.

[e] ND, not done.

RESULTS

The requirement for *vif* during HIV-1 replication in CD4$^+$ T lymphocytes. The effect of *vif* on HIV-1 replication in five human CD4$^+$ T-cell lines was examined by using a pair of HXB2 viruses that were isogenic except for the ability to express *vif*. The *vpr*, *vpu*, and *nef* genes of this strain are defective (5, 17). For these experiments, infection was initiated by transfection of the T-cell lines with equivalent amounts of either *vif*-positive or *vif*-negative HXB2 DNA. The cell lines used include the CD4$^+$ cell lines SupT1 (26), C8166 (23), Jurkat (33), CEM (8), and H9 (18). C8166 cells are CD4$^+$ T cells that are immortalized by human T-cell leukemia virus type I but do not produce the structural proteins of the virus (23). These five T-cell lines can support the growth of HIV-1 (7, 18, 22, 27, 29). These cell lines express different levels of CD4 and have differing syncytium-forming abilities. The relative amounts of CD4 expressed on the cell surface were determined by fluorescence-activated cell sorter (FACS) analysis and are given in Table 1. The relative syncytium-forming abilities of the cell lines were determined by cocultivation of each of the T-cell lines with COS-1 cells expressing HIV-1 envelope glycoproteins (Table 1).

The replication of the *vif*-positive and *vif*-negative HXB2 viruses was monitored by measuring the level of reverse transcriptase activity in the culture supernatants (Fig. 1). All five cell lines were able to support the growth of HIV-1 initiated by transfection of the *vif*-positive HXB2 DNA. High levels of virus production were detected in supernatants of cultures transfected with the *vif*-positive HXB2 DNA by 7 days postinfection in all five cell lines. In contrast, very low levels of viral reverse transcriptase activity, <1,100 cpm/ml, were released into the supernatants of cultures of

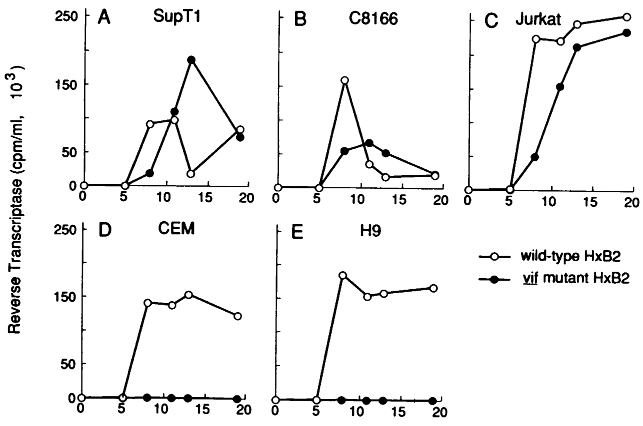

FIG. 1. Replication of *vif*-positive and *vif*-negative HXB2 viruses in CD4+ human T-cell lines. Reverse transcriptase activity in the supernatants of T-cell cultures transfected with 10 μg of pHXB2 (open circles) or pHXB2ΔAvr (solid circles) plasmid DNA was measured as previously described (21). The results are typical of those seen in at least three independent experiments.

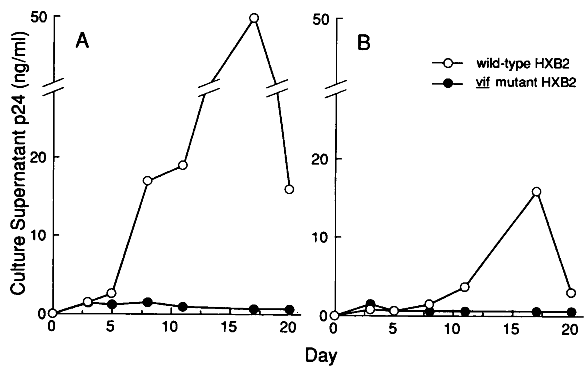

FIG. 2. Replication of *vif*-positive and *vif*-negative HXB2 viruses in cultures of peripheral blood T lymphocytes. Approximately 200,000 T cells were infected with 5,000 cpm of reverse transcriptase units of *vif*-positive (open circles) or *vif*-negative (solid circles) HXB2 virus stock produced in either SupT1 (A) or Jurkat (B) cells. Virus replication was monitored by measuring p24 antigen production in the culture supernatants. The results are typical of those seen in four independent experiments.

CEM and H9 cells transfected with the *vif*-negative HXB2 DNA. Detectable amounts of the *vif*-negative virus were produced by cultures of SupT1, C8166, and Jurkat cells transfected with the *vif*-negative HXB2 DNA. However, the onset of replication of the *vif*-negative virus in these cell lines was delayed by 3 to 5 days compared with the onset of replication in cultures transfected with the *vif*-positive virus DNA. The higher peak level of reverse transcriptase activity in the SupT1 cultures infected with the *vif*-negative virus probably resulted from delayed cell killing, as a consequence of delayed virus replication. The cytopathic effect of virus replication in the cultures as judged by the number and size of syncytia and by the decrease in total cell number was similar in cultures infected with the *vif*-positive and the *vif*-negative virus when levels of viral reverse transcriptase activity in the culture supernatants were similar. Similar results were obtained when the same experiment for which the results are shown in Fig. 1 was done by using the HXB2*vif*stop mutant virus, in which *vif* is truncated by two in-frame stop codons (data not shown).

The effect of *vif* on the establishment of infection in peripheral blood T cells was examined. Primary T-lymphocyte cultures were infected with equivalent amounts of *vif*-positive or *vif*-negative virus stock made in transfected Jurkat or SupT1 cultures. As shown in Fig. 2, T-cell cultures infected with the *vif*-positive virus produced in either SupT1 or Jurkat cells produced peak levels of p24 antigen production of 50 or 16 ng/ml, respectively. In contrast, the amount of p24 antigen released following infection with the same amount of *vif*-negative HXB2 virus was undetectable or nearly undetectable.

Effect of *vif* during a single round of virus replication. Detectable virus replication in CD4+ T-cell cultures initiated by transfection requires multiple rounds of virus replication. The requirement for multiple rounds of replication obscures the magnitude of the difference in infectivity of the *vif*-positive and *vif*-negative virus in a single round of infection. To obtain a quantitative measure of the effect of *vif* during a single round of infection, a transient *trans*-complementation assay was used (12). Cotransfection of a plasmid, pHXBΔenvCAT, that contains an envelope-defective HXB2 virus expressing the bacterial CAT gene (31) with a plasmid that expresses the HIV-1 envelope gene results in the production of a virus that can initiate a single round of infection in susceptible CD4+ cell lines (12). The infection is limited to a single round as the packaged viral genome is defective for production of the *env* protein. The efficiency of a single round of infection is determined by measurement of the level of CAT enzyme activity in the infected culture.

The effect of *vif* during a single round of virus replication in the different CD4+ T-cell lines was measured by cotransfection of the HIV-1 envelope expressor DNA and either the *vif*-positive or *vif*-negative envelope-defective CAT virus DNA (12, 19). In this transient complementation assay, the amount of virus present in transfected T-cell culture supernatants is too low to initiate cell-free infection. The levels of p24 viral antigen detectable in the transfected T-cell supernatants at 48 to 72 h after transfection are <1 ng/ml, and most of the virus transmission results from cell-to-cell spread. The data in Table 1 show that the level of CAT enzyme activity 9 days after cotransfection was greater for cells transfected with *vif*-positive CAT virus DNA than for those transfected with *vif*-negative CAT virus DNA. The ratio of CAT enzyme activity in cultures transfected with *vif*-positive CAT virus DNA to that in cultures transfected with *vif*-negative CAT virus DNA was 2:1 in SupT1 and C8166 cells, 3:1 in Jurkat and CEM cells, and 6:1 in H9 cells (Table 1). To determine whether *vif* can act in *trans* to increase the efficiency of infection in the single-step assay, 1 μg of the *vif*-negative envelope-defective CAT virus DNA was cotransfected into CEM cells with both 1 μg of the *env* expressor plasmid and 8 μg of a *vif* expressor plasmid. The latter plasmid expresses the HXB2 *vif* protein under the control of a simian virus 40 promoter in the pSVL expression vector (Pharmacia). The pSVL plasmid without *vif* sequences was used as a control. The level of CAT enzyme activity made by the *vif*-negative CAT virus in the presence of *vif* expressed in *trans* was similar to that made by the *vif*-positive CAT virus (data not shown). The level of CAT enzyme activity was not increased by cotransfection with the control plasmid.

Effect of *vif* during virus production. The effect of *vif* during virus production and virus entry was examined to determine which stage of the virus life cycle is affected by *vif*. Different cell lines were used as either virus producer or target cells during a single round of virus replication by using the single-step assay described above. For the first experiment, *vif*-positive and *vif*-negative CAT viruses were produced in the monkey kidney cell line COS-1. The data in Table 1 show that the same amount of CAT enzyme activity was present in cells infected with *vif*-positive and *vif*-negative CAT virus made in COS-1 cells. No difference in the levels of CAT enzyme activity was observed by using *vif*-positive and *vif*-negative CAT virus produced in COS-1 cells when the amount of virus used was either decreased by 10- or 20-fold or increased by 6- to 12-fold or when the level of virion-associated HIV-1 envelope glycoproteins was reduced by decreasing the amount of transfected envelope expresser plasmid DNA to 0.1, 0.25, 0.5, or 1.0 μg (9) (data not shown).

The possibility that the lack of effect of *vif* on the infectivity of viruses produced in COS-1 cells was due to the absence of CD4 was examined by including 5 μg of a CD4 expresser plasmid, pCDNA1.CD4 (25), with the envelope expresser plasmid and the *vif*-positive or *vif*-negative envelope-defective CAT virus DNAs in the transfection. Coexpression of CD4 did not alter the lack of effect of *vif* on the infectivity of viruses produced in COS-1 cells (data not shown).

Similar experiments were done by using virus produced in $CD4^+$ T-cell lines rather than COS-1 cells. The two cell lines used were SupT1 and CEM. The *vif*-negative HXB2 virus replicates well in SupT1 cultures but poorly in CEM cultures. For these experiments, the *vif*-positive or *vif*-negative envelope-defective CAT virus DNA was cotransfected with the envelope expresser DNA into either SupT1 or CEM cells and the ability of the virus produced to infect either the same cell line or the other cell line was examined. Infection was initiated by cocultivation of 2×10^6 transfected virus producer cells with 2×10^7 target cells. The results in Fig. 3A show that the levels of CAT enzyme activity were 1.5- to 1.9-fold greater when *vif*-positive CAT virus produced by SupT1 cells was used to infect either SupT1 cells or CEM cells than when similarly produced *vif*-negative CAT virus was used. By contrast, 5 to 9 times the level of CAT enzyme activity was produced by *vif*-positive CAT virus than by *vif*-negative CAT virus when the virus was made in CEM cells and used to infect either SupT1 or CEM cells.

The preceding experiment measured the effect of *vif* under conditions in which most of the virus transmission occurs by cell-to-cell spread. The results indicate that the effect of *vif* depends on the cell line in which the virus is made rather than the cell line that is subsequently infected. This possibility was examined by using replication-competent virus stocks produced in cultures of SupT1, C8166, Jurkat, or CEM cells. The efficiency of cell-free transmission of the *vif*-positive and *vif*-negative HXB2 virus stocks produced in different T-cell lines was compared. A low infectious dose (approximately 0.05 infectious unit per cell) of *vif*-positive and *vif*-negative virus stock produced in either SupT1, C8166, Jurkat, or CEM cells was used to initiate infection in three different T-cell lines. The efficiency of cell-free virus transmission was compared by measuring the level of reverse transcriptase activity in the culture supernatants on days 2, 4, and 8 after infection. The data shown in Fig. 3B reflect the earliest time points at which virus replication could reliably be measured. *vif* enhanced the cell-free infectivity of virus stocks produced in SupT1 cells 1.4- to 1.6-fold. In contrast, the relative cell-free infectivity of virus stocks

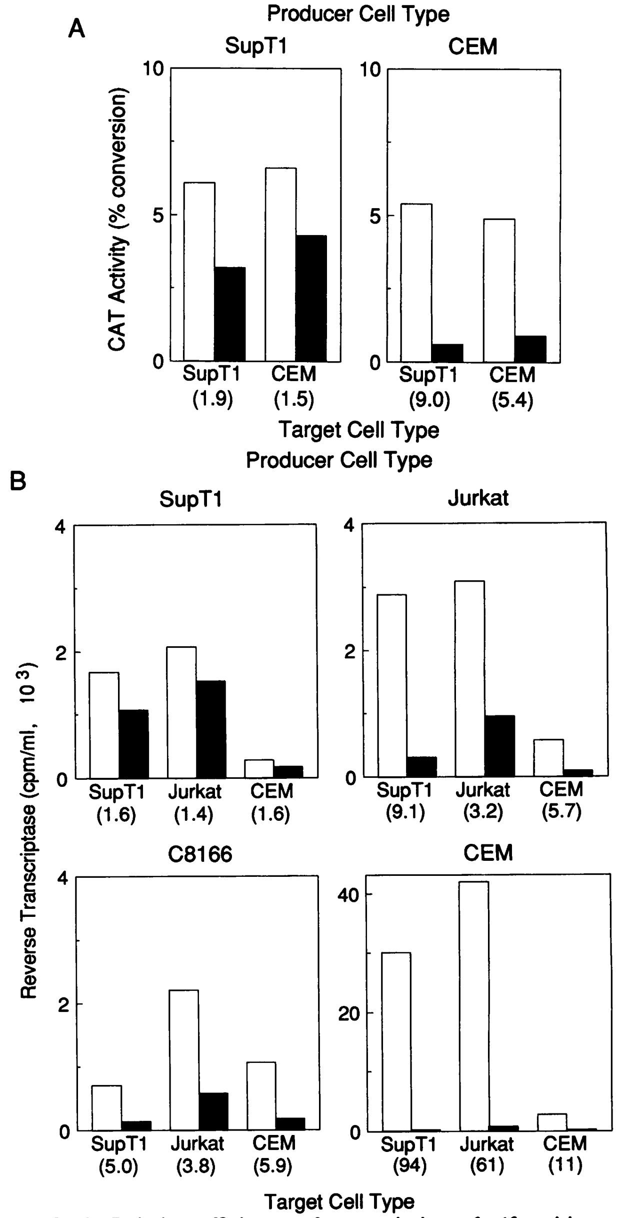

FIG. 3. Relative efficiency of transmission of *vif*-positive and *vif*-negative viruses produced in different T-cell lines. (A) Relative efficiency of a single round of replication of *vif*-positive and *vif*-negative virus produced in SupT1 (left) or CEM (right) cells following cotransfection with an HIV-1 envelope expresser plasmid and either pHXBΔenvCAT (open bars) or pHXBΔAvrΔenvCAT (solid bars). Values shown represent the CAT activity (% conversion) 9 days after cocultivation of 2×10^6 transfected producer cells with 2×10^7 untransfected target cells. The values shown in parentheses under the target cell type indicate the fold enhancement of virus transmission for the *vif*-positive virus relative to that of the *vif*-negative virus. (B) Relative efficiency of cell-free virus transmission of *vif*-positive (open bars) and *vif*-negative (solid bars) HXB2 virus stocks produced in different T-cell lines. $CD4^+$ T cells (5×10^6) were infected with 5,000 cpm of reverse transcriptase units of *vif*-positive or *vif*-negative HXB2 virus stock for 16 h. The medium was changed at 16 and 72 h after infection, and reverse transcriptase activity in the culture supernatant was measured on day 4 (SupTl, C8166, and Jurkat virus stocks) or day 8 (CEM virus stocks). The values shown in parentheses under the target cell type represent the fold enhancement of the efficiency of cell-free virus transmission of the *vif*-positive virus stock relative to that of the *vif*-negative virus stock.

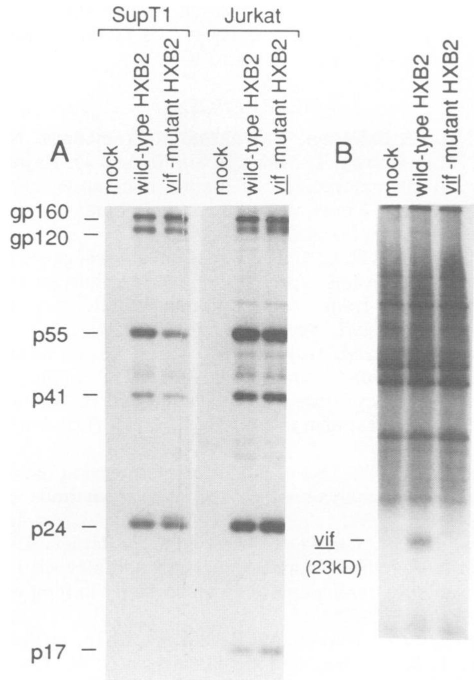

FIG. 4. Viral proteins in *vif*-positive and *vif*-negative HXB2-infected Jurkat and SupT1 cultures. Cultures were metabolically labelled with [^{35}S]cysteine and [^{35}S]methionine for 16 h, and cell lysates were immunoprecipitated with an AIDS patient's serum (A) or rabbit antiserum raised against a *vif* peptide (B) 14 to 21 days after transfection of cells with no DNA (lane labelled mock), 10 μg of pHXB2 (lane labelled wild-type HXB2), or pHXB2ΔAvr (lane labelled *vif* mutant HXB2) and analyzed by sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis (27). The markers on the left indicate the positions of the viral *env* and *gag* proteins.

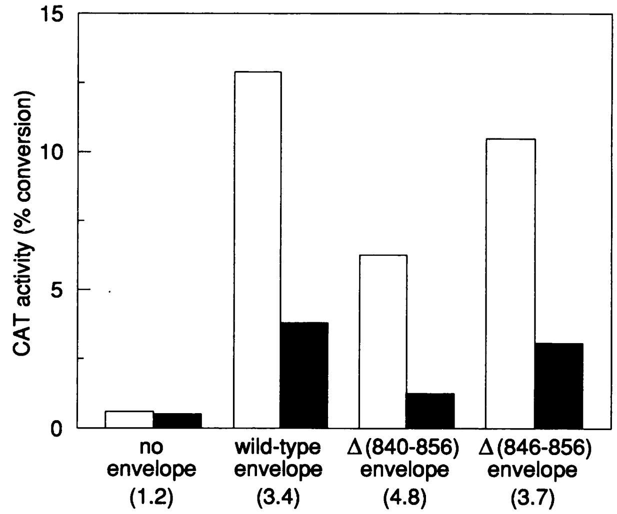

FIG. 5. Relative infectivity of *vif*-positive and *vif*-negative CAT viruses containing carboxy-terminal deletions in gp41 during a single round of virus replication. Values shown represent CAT activity (% conversion) in CEM cell lysates 9 days after cotransfection with 5 μg of PSVIIIenv, pSVIIIenvΔ840-856, or pSVIIIenvΔ846-856 and 5 μg of pHXBΔenvCAT (open bars) or pHXBΔAvrΔenvCAT (solid bars). The values shown below the transfected envelope expressor plasmid represent the fold enhancement of transmission of the *vif*-positive virus relative to that of the *vif*-negative virus for each wild-type or mutant envelope glycoprotein.

produced in C8166 or Jurkat cells was enhanced 3.2- to 9.0-fold by the presence of *vif*. *vif* enhanced the cell-free transmission of virus produced by CEM cells 60- to 90-fold. The effect of *vif* on cell-free transmission was similar when SupT1, Jurkat, or CEM cells were used as targets.

Effect of *vif* on viral protein synthesis and processing. The effect of *vif* on the synthesis and processing of HIV-1 *env* and *gag* proteins in different cell lines was examined. Cell lysates from *vif*-positive and *vif*-negative HXB2-infected Jurkat and SupT1 cells were immunoprecipitated following metabolic labelling with [^{35}S]methionine and [^{35}S]cysteine by using an AIDS patient's serum (27). There was no difference in the levels of *env* or *gag* proteins in the *vif*-positive and *vif*-negative HXB2-infected SupT1 and Jurkat cultures (Fig. 4A). Similar results were observed for C8166 cultures infected with the *vif*-positive or *vif*-negative virus (data not shown). Viral proteins could not be detected in the CEM cultures infected with *vif*-negative virus by immunoprecipitation, presumably because of the low levels of virus replication.

A rabbit peptide antiserum raised against amino acids 170 to 184 (TEDRWNKPQKTKGHR) of the *vif* protein (American BioTechnologies, Inc.) was used to immunoprecipitate the 23-kDa *vif* protein in cell lysates of infected cultures (Fig. 4B). The 23-kDa *vif* protein was detected by immunoprecipitation in *vif*-positive but not in *vif*-negative HXB2-infected Jurkat cell lysates (Fig. 4B). Similar results were observed in SupT1, C8166, CEM, and COS-1 cell lysates (data not shown).

Carboxyl-terminal deletions in gp41 do not alter the effect of *vif*. The results of a previous study suggested that a region within the last 15 residues at the carboxyl terminus of gp41 might be one of the targets of HIV-1 *vif* (11). To determine whether the carboxyl terminus of gp41 is required for the effect of *vif* on viral replication, CEM cells were transfected with the HIV-1 envelope expresser plasmid or a mutant HIV-1 envelope expressor plasmid deleted for sequences that encode the carboxyl-terminal 17 amino acids of gp41 (pSVIIIenvΔ840-856 or pSVIIIenvΔ846-856) (9, 17) together with the *vif*-positive or *vif*-negative envelope-defective CAT virus DNAs. The efficiency of a single round of virus replication was measured as described above. Deletion of residues 840 to 856 or 846 to 856 did not alter the effect of *vif* during a single round of virus replication in CEM cells (Fig. 5).

DISCUSSION

The data show that the requirement for *vif* during virus replication differs among established human CD4+ T-cell lines. In the absence of *vif*, the onset of virus replication in SupT1, C8166, and Jurkat cells was delayed but peak levels of virus replication were similar. In contrast, virus replication was nearly undetectable in H9 and CEM cultures infected with the *vif*-defective virus. Likewise, in peripheral blood T lymphocytes, *vif* was essential for establishing HIV-1 infection. These results suggest that *vif* is likely to play an essential role during HIV-1 infection in vivo, consistent with the results of previous studies (1, 7, 16).

In cells in which *vif* enhances viral infectivity, *vif* is required at the time of virus production. In CEM cells, *vif* was required for production of virus capable of initiating cell-free infection in all cell lines studied. By contrast, *vif*-defective virus produced by COS-1, SupT1, C8166, and

Jurkat cells could initiate infection in CEM and H9 cells. Similar levels of the *vif* protein were made in the different cell lines, indicating that there were no significant differences in *vif* mRNA translation among the cell lines used in this study. The requirement for *vif* in different cell lines did not correlate with the level of CD4 expression. The lack of requirement for *vif* during virus production in COS-1 cells was not affected by reducing the level of the envelope glycoproteins, indicating that the lack of an effect in COS-1 cells was probably not merely a consequence of overexpression of *env*. It is noteworthy that the cell lines with the greatest requirement for *vif*, H9 and CEM, have the lowest syncytium-forming abilities. Further studies are required to determine the significance of this observation.

These results demonstrate that the *vif* protein affects the late phase of the virus life cycle. The demonstration that the *vif* mRNA is expressed late in the virus life cycle (10, 24) and the finding that *vif* is present in infected cells but not in virions (2, 7, 13, 15, 22, 27) also indicates that the *vif* protein acts during the late phase of the virus life cycle. The finding that *vif* enhances viral infectivity during virus production and does not affect transcription, translation, or virus release (7, 27, 29) suggests that *vif* may be important during the processing of virion proteins, virion assembly, or virion maturation. The results of this study also suggest that *vif* can compensate for cellular factors required for production of infectious virus particles that are present in some cell lines, such as SupT1, C8166, and Jurkat, but are absent in others, such as CEM and H9.

On the basis of previous studies, it has been suggested that *vif* is essential for efficient cell-free infectivity (7, 29). This study demonstrates that *vif* is not required for efficient cell-free transmission of virus in all cell lines. This study also confirms the results of previous studies (7, 22) which suggested that cell-to-cell transmission is enhanced by *vif* on the basis of the observation that *vif* enhanced a single round of infection in transfected T-cell lines under conditions in which most of the virus transmission occurs by cell-to-cell spread. The role of *vif* during cell-free and cell-to-cell transmission in different types of naturally infected target cells in vivo remains to be determined. Further studies are required to determine whether the enhancement of viral infectivity by *vif* results in a subsequent increase in the efficiency of virus entry, reverse transcription, or events after reverse transcription.

A region of structural homology between HIV-1 *vif* and a family of cysteine proteases was described in a previous study (11). The investigators for that study demonstrated that a specific inhibitor of cysteine proteases impairs a *vif*-dependent modification of the HIV-1 envelope glycoproteins and proposed that a region within the last 15 residues at the carboxyl terminus of gp41 might be a target for *vif* function. The results of the present study demonstrate that this region of the carboxyl terminus of gp41 is not required for the effect of *vif* on virus replication, indicating that it is unlikely that this region of gp41 is an important target for *vif* function.

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